Protein Radicals, Regulations, and Cancer

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Abstract

How far is life a molecular or electronic solid-state phenomenon? Primitive "vegetative" functions are performed by soluble protein molecules in molecular reactions. Complex "animal" functions are performed by insoluble structures built of protein biradicals, doped by the incorporation of electron acceptors.

Life is a miracle, but even miracles must have their underlying mechanism. The archives in which the basic blueprints of this mechanism are preserved and "xeroxed" are the nucleic acids, while the business of life is carried on by the proteins. Our paper will be concerned solely with the latter. Life has unique qualities, and so proteins must have unique qualities too. The object of our paper will be to find these.

Life's history consists of two chapters, divided by the appearance of light and oxygen. The first dark and anaerobic part we will call the α, the second anaerobic and light part the β period. Life was born on a dark and airless globe, covered by dense water vapor. It began to develop and differentiate when, due to cooling, the water vapor condensed and light could reach the surface of the globe.

The protein was created in the α period by linking amino acids together by peptide bonds to long chains which then were folded up in a way which brought certain atomic groups of the side chains close together and lent a catalytic activity to the protein molecule, enabling it to perform simple reactions, as making and breaking of bonds. It enabled the protein to break up foodstuff molecules in a way which liberated a small part of their energy in a process we call "fermentation." These simple "vegetative" functions were performed by single protein molecules, or an assembly of such molecules, dissolved in molecular dispersion.

The complex "animal" functions were developed in the β period by lending a high degree of reactivity to these protein molecules and linking them together in a specific way to complex insoluble structures with an integrated function. "Structures" by definition are insoluble. This building of structures underlied differentiation, which reached its peak in the thinking of man.

When Nature discovers a new principle and builds a new system on it, she does not throw the old one out, but builds the new on top. So when life, in the β period, built up the system of structures, she did not throw the system of dissolved molecules out, but continued to perform the simple vegetative funetions with them.

It is well to keep this duality of simple vegetative and complex animal functions in mind, the first performed by the system of dissolved molecules, and the second

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performed by structures. The disregard of this duality has delayed our understanding of the living state and led to a serious misunderstanding [1].

It was four decades ago [2] that we felt that the subtle reactivity of complex biological reactions could not be brought about by the simple reactions of single clumsy macromolecules. This made us propose that proteins may be semiconductors. Our proposition was unanimously rejected and remained so to the present. The main, apparently decisive argument was that none of the great number of proteins isolated in crystalline condition showed any signs of semiconductivity. It was not noticed that any protein which can be crystallized has to belong to the dissolved kind. The structures which needed semiconductivity could not be crystallized. So only dissolved proteins were studied, and the structures, not being crystallizable, were disregarded. The difficulty was solved by calling the insoluble part of the extracted tissues "the residue" and sending it down the drain.

The main object of our paper will be to find the specific features of these structures which made differentiation, and the development of "higher" animal functions possible.

When life originated there was no oxygen near the surface of the globe, and the atmosphere had to be reducing, being dominated by electron donors, that is, molecules tending rather to give off than to take up electrons. No strong electron acceptors could persist. The orbitals of the protein molecules had to be electronically saturated, occupied by pairs of localized electrons. The whole molecule had to be unreactive and dielectric.

Such closed-shell molecules can be transformed into highly reactive free radicals by taking an electron out of them. When taking out an electron, an electron pair has to be uncoupled. An uncoupled electron, an electron hole, and a partially occupied orbital have to be created, making out of the unreactive molecule a highly reactive radical with delocalized electrons.

Electrons can be taken out of a molecule by other molecules, acting as "electron acceptors," which, having a low-lying unoccupied orbital, can accommodate an additional electron. This process of transferring an electron from one molecule to the other is "charge transfer." It makes out of the electron donor a positively, and out of the electron acceptor a negatively charged radical. (Charge transfer has to be distinguished from oxidation in which electrons are transferred from one molecule to the other pairwise, in which no electron pairs are separated and no radicals are created, a radical being a molecule with an uncoupled electron.)

When light appeared on this globe, life used this light's energy to separate with it the elements of water. The hydrogen it fixed by linking it to carbon, the oxygen it released as O_2. Oxygen is a strong electron acceptor, so henceforth the atmosphere was no more dominated solely by electron donors, but was dominated by a new parameter, the D/A quotient, the relation of electron donors to electron acceptors. This opened the possibility of taking electrons out of protein and transforming it into the free radical state, creating the unbalanced forces which could bind molecules together to structures, and creating the electronic mobility which was needed to integrate the function of the structures, endowing them with the qualities of "solid state."

Charge transfer leads to the formation of two charged radicals. Charge radicals are so reactive that many biologists thought their persistence incompatible with life. The research of our laboratory shows that Nature achieves the same end, the transformation of unreactive protein molecules into reactive radicals in a somewhat different way. What she does is to incorporate the whole acceptor molecule covalently into the protein molecule, and *then* transfer the electrons of proteins onto it. Both the positive and negative charge being generated inside the protein molecule, the resulting protein biradical has no net charge and will not endanger life by its perturbations. Such an intramolecular charge transfer is a hitherto unknown process which leads to very specific electronic structures. The whole process is closely analogous to the "doping" by which electronic industry generates its semiconductors. Nature discovered "doping" eight hundred million years before man.

Before discussing our simple experiments, we will have to say a few words about the acceptor which could be used to desaturate the protein electronically. The oxygen molecule O=O consists of two O atoms linked by a double bond. It is unfit to serve as electron acceptor in charge transfer because it is a di- or tetravalent acceptor, and so it will take over electrons pairwise, and will "burn" instead of producing free radicals. Not so if the O atom is linked to carbon, instead of being linked to another O. In this case the resulting C=O, the carbonyl formed, is a monovalent acceptor. Being a small atomic group, it cannot easily accommodate an additional whole electron and is thus a "weak acceptor." It can be made into a "strong" acceptor by placing another C=O at its side. In this case the π electron pools of the two conjugated double bonds fuse to a big π pool which is a "strong" but still monovalent acceptor that easily takes up a whole electron. The simplest dicarbonyl is the dialdehyde glyoxal, OCHHCO, the first derivative of which is the ketoaldehyde, methylglyoxal, CH_3COCHO (MG).

That we have not trodden here on the path of meaningless speculation is indicated by the fact that more than sixty years ago a most active and apparently ubiquitous enzymic system, the "glyoxalase," was discovered, which can transform MG into D-lactic acid. Nature does not indulge in luxuries, and if there is such a highly active and widely spread enzymic system, it must have something very important to do, but nobody knew what, neither MG nor D-lactic acid lying on a metabolic highway. Should MG, or another closely related dicarbonyl, serve as acceptor in the electronic desaturation of protein, this would give a satisfactory explanation.

If protein donates an electron to MG, this has to be one of the two electrons described as the nonbonded electrons of nitrogen. Proteins being very complex, we started studying this charge transfer reaction using, instead of protein, the simplest amine, methylamine (MA). We started with mixing a 0.1*M* aqueous solution of MA and MG. What could be expected to happen was the formation of a yellow Schiff base:

$$CH_3—NH_2 + HC=OC=OCH_3 \rightleftharpoons CH_3—N=CHC=OCH_3 + H_2O$$

the yellow color being due to the chromogenic C=N group. The reaction

probably proceeds in two steps. First, one of the H's of N is transferred onto the O aldehydic O, and then a molecule of water is eliminated and a double bond is established between C and N. In the Schiff base formed, the amino N and the ketonic O become members of a single conjugated system in which the non-bonded electron of the N can freely move to the ketonic O, and can become located on its low-lying orbital.

An unexpected result was obtained when the same experiment was repeated with alcoholic solutions of MG and MA. In this case a deep purple color was developed which was due to a narrow absorption peak, at 475 nm.

The difference between the yellow and purple substances is still under discussion. What is of import here is that the purple substance was found to be relatively insoluble in acetone, while both the MA and MG were freely soluble in this solvent. This opened the way to the isolation of the purple substance. When a 0.5*M* acetone solution of MA and MG was mixed, a colorless precipitate was formed which, in seconds, turned purple. In the electron spin resonance spectroscope it gave a strong signal with a rich hyperfine structure. Evidently it was a radical formed by the transfer of an electron from amine to the carbonyl. Pohl measured the MW. His data suggest a tetramer of the Schiff base [3]. It was surprising to find that while in water no purple complex was formed; the substance formed in acetone was soluble and stable in water. It was not split up by strong acid, which indicated the amine and diketone were covalently linked, were part of a single biradical.

It is a long cry from MA to protein, and the question was whether proteins gave similar reactions. For experimental material we chose the protein casein. With Jane McLaughlin we suspended granular casein in methanol to which 10% of the commercial 40% MG was added. Then we incubated overnight. The next day we found the originally white protein brown. It assumed the color of a bloodless liver. In the electron spin resonance spectroscope, in the hands of Pohl and Gascoyne [4] it gave a strong signal with a double peak, as could be expected from a biradical. The signal centered around g 2.00. Similar results were also obtained with aqueous suspensions of casein, incubated with MG, precipitated isoelectrically, and washed with alcohol. The color could not be eliminated by repeated precipitation. These experiments thus show that proteins, similar to simple amines, can form stable biradicals with electron acceptors. They can do this by incorporating the acceptors covalently into their molecule and then transferring electrons onto them. The brown color of liver is the color of its protein biradicals.

As is generally known, live tissues such as freshly isolated liver give electron spin resonance signals. The question was whether these signals are due to metabolic free radicals or to protein radicals which make part of the structures. To eliminate metabolism and its soluble free radicals, the livers of mice were blendored in an ice-cold half-saturated ammonium sulfate solution which eluted soluble proteins and radicals. The insoluble structures were separated on the centrifuge. In the ESR spectroscope they gave signals similar to those of MG-treated casein, which has an increased electronic conductivity [5]. All this, taken together, indicated that the brown color and electron spin resonance signal of

the liver are due to the protein radicals out of which its structures are built. The brown color indicates electronic delocalization and semiconduction. The number of the spins in the casein, treated with MG, was found to be in the range of unity, one spin per casein molecule, which suggested that the acceptor was attached to a specific amino group which did not belong to a side chain but belonged to the whole protein molecule. There is but one such group: the terminal amino group. This makes it probable that the structural proteins of the liver have an acceptor covalently attached to their terminal NH_2, which makes them into biradicals. If the electron taken out by the acceptor is derived from the valence band of the donor, then the charge transfer has to make the protein conductant in the ground state.

To our knowledge no such electronic structures have been described yet in proteins. They seem to represent the main form of structural proteins which have an active function. Needless to say, there are also protein structures which have a static passive function which demands no semiconduction and no free radicals. Such are tendons, fasciae, valves, cartilage, the inner surface of big arteries, or the supporting matter of the brain, which are all white, colorless.

The described relations shed a new light on the nature of the living state. We succeeded but lately to detach the acceptors from the protein. Its identification is being attempted.

From the study of monoamines we shifted to the study of diamines, starting our experiments with ethylenediamine, $H_2NCH_2CH_2NH_2$. This substance seemed especially interesting because it has in its backbone the same atomic sequence as protein: NCCN. Mixing an aqueous solution of ED with that of MG, a slowly appearing yellow color indicated the formation of a Schiff base. On addition of glutathione an intense purple color appeared, accompanied by an electron spin resonance signal [6]. The color was again due to a narrow absorption peak at 475 nm. The reaction was not specific for glutathione, and was shared by other SH sulfhydryls, like *N*-acetyl cysteine. As found by Pohl [3], the color was the strongest if the MG was isomolar with the SH. Excess SH strongly inhibited the reaction. Evidently, the reaction was a reaction of the SH group. SH acted as catalyst. It was not used up in the reaction.

With this experience in hand we went over the reactions of monoamines again and found that they all were catalyzed by SH. Even the formation of the yellow Schiff base was speeded up. Since glutathione is present in all animal cells, the reactions of MG and amines *in vivo* have to be catalyzed by it. For these reactions the MG does not act as such, but acts in the form of its hemimercaptale formed with SH-glutathione. This is the second known catalytic action of glutathione, the first being the activation of the glyoxalase.

To make life perennial, in the α period the living system had to proliferate as fast as conditions permitted. In the differentiated β state this proliferation had to be arrested in the interest of the harmony of the whole. Cell division had to be subjected to regulation. In this period the semisolid structures had to impede cell division. So if division was called for, these structures had to be partly disassembled, the whole cellular edifice loosened up, the protein radicals relieved of their acceptor and returned to the molecular state. The α–β transition had

to be reverted, leading the cell back toward the α state. Part of the oxidative mitochondria being broken up, the dividing cell has to rely in an increased measure on fermentation for the production of energy. After it completed its division, it had to build up its β state again. All this is not merely a playing with ideas, a *jeux d'esprit*. Should the dividing cell find the road of return to the β state perturbed, then it has to persist in the proliferative α state, and tumor results.

It seems logical that for the arrest of cell division, the cell should have used the same substance by which it induced the β state: the dicarbonyls. Egyud and this author [7] found that methylglyoxal, which induces the β state, can also arrest proliferation. It arrested cell division in all tested materials in a low concentration, reversibly, without harming the cell. The C=O group seems to be the universal tool of Nature for the arrest of proliferation. For the reversible arrest she uses the mild aliphatic CO's. For irreversible arrest, which is killing, she uses the potent aromatic C=O's, quinones. Plants defend themselves against bacteria by producing quinones. The carbonyls arrest cell division partly by helping to build solid structures and partly by neutralizing the highly active SH's, which are indispensable for cell division and generate the high electron tension needed for proliferation.

We want to conclude this paper with asking: has all this anything to do with cancer? As any dividing cell, the cancer cell has all the qualities of the α state—it is actually a cell stuck in the α state. Whatever may have been the oncogenic agent, the cancer cell, excepting melanomas, has no color, has a loose structure, is undifferentiated. The central event of the α–β transformation is the incorporation of the electron acceptor, the transformation of protein molecules into radicals. This reaction seems to be in the focus of carcinogenesis. The details of the chemical mechanism of this reaction still await elucidation.

To press all this into a nutshell, we can say that life is based on two miracles. Miracle one was the creation and folding of the protein molecule. Miracle two was the transformation of this molecule into a highly reactive radical. The cancer cell seems to be unable to perform miracle two.

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